

AChR-Specific Immunosuppressive Therapy for MG

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Grant support from the Muscular Dystrophy Association

Myasthenia Gravis (MG) and Experimental Autoimmune Myasthenia Gravis (EAMG)

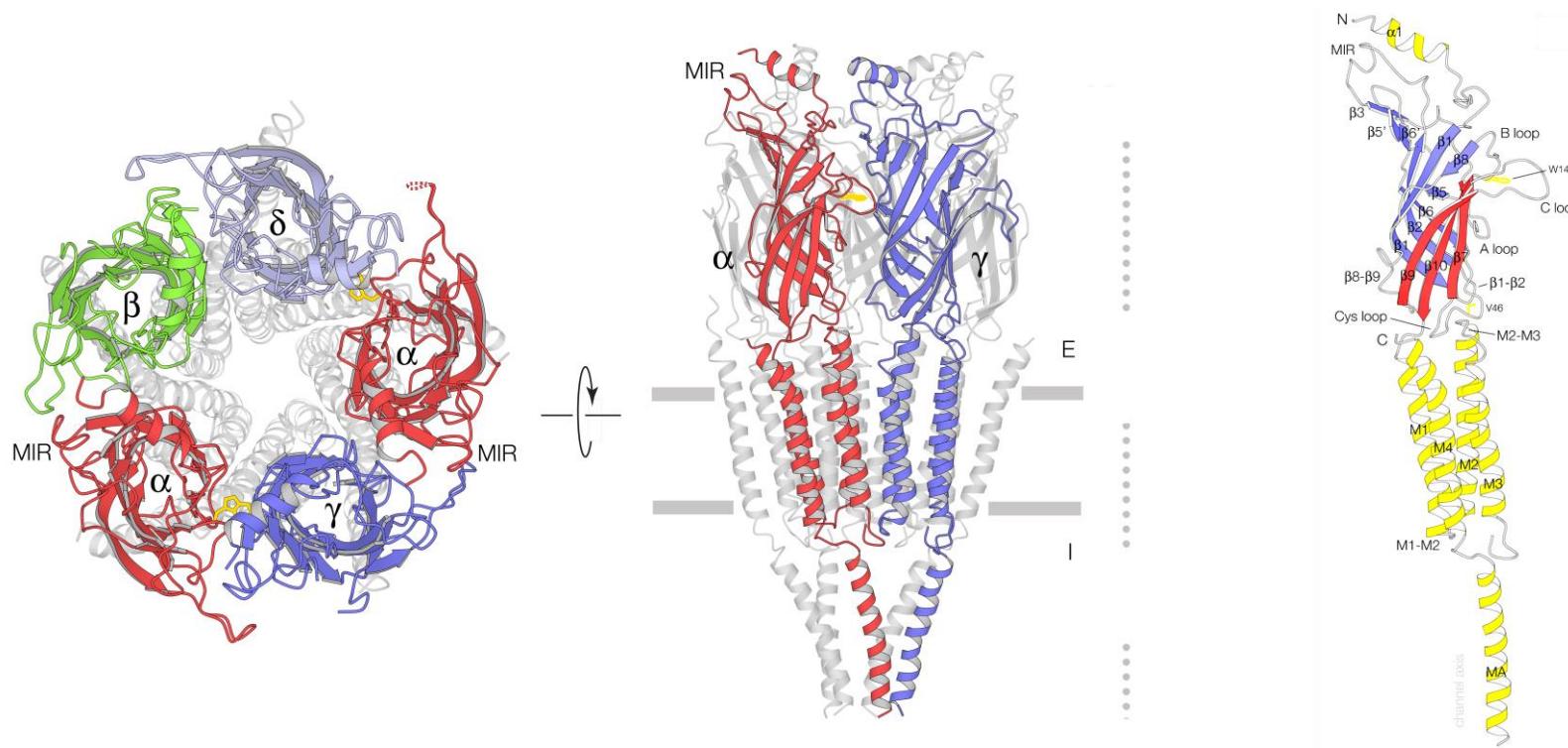
- MG is mediated by autoantibodies to muscle $\alpha 1^*$ AChRs
 - Characterized by weakness and fatigue
 - Autoantibodies impair neuromuscular transmission
 - Complement-mediated focal lysis destroys AChRs and disrupts synaptic morphology
 - Crosslinking of AChRs by antibody destroys AChRs through increased turnover
- EAMG is the animal model of MG
 - Usually induced by immunizing with $\alpha 1^*$ AChRs from *Torpedo* electric organ
 - Can be induced efficiently by a chimera of human $\alpha 1$ main immunogenic region in *Aplesia* AChBP
 - Can be induced inefficiently by native AChBP
 - Can be induced inefficiently by bacterially-expressed extracellular domain of $\alpha 1$ subunits

The Main Immunogenic Region (MIR)

- The MIR is a conformation-dependent region at the extracellular tip of muscle AChR α 1 subunits, defined by competitive binding of mAbs.
- It is the target of half or more of autoantibodies in MG and EAMG.
- mAbs to the MIR have all of the pathological activities of serum autoantibodies to AChRs: bind *in vivo*, fix complement, crosslink AChRs causing antigenic modulation, and passively transfer EAMG.
- Its conformation depends on interaction of the N-terminal α helix (α 1-14) and the MIR loop (α 67-76).
- A chimera of human α 1 (1-30, 60-81) with *Aplesia* acetylcholine binding protein (AChBP) binds conformation-dependent mAbs to the MIR with high affinity and binds autoantibodies from humans, dogs, and cats with MG (Luo et al. (2009) J. Neuroscience 29:13898).

Structure of Skeletal Muscle-Type AChRs from *Torpedo* Electric Organ

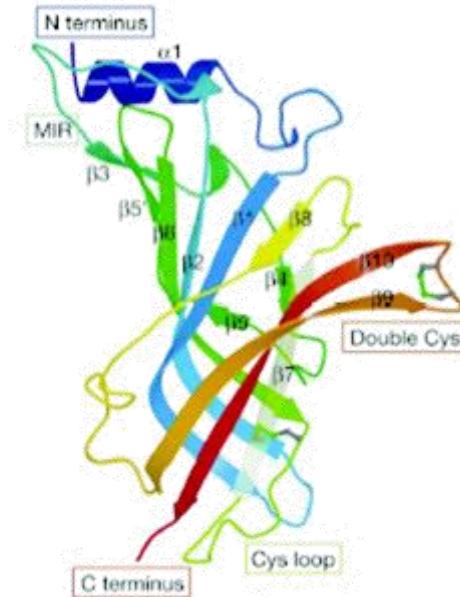
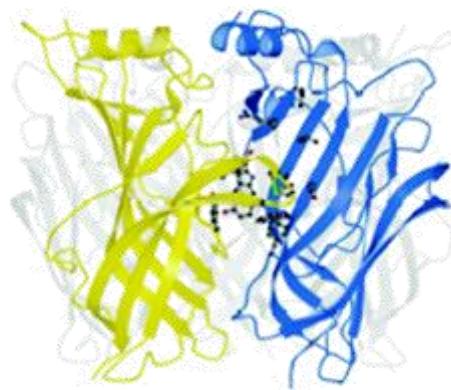
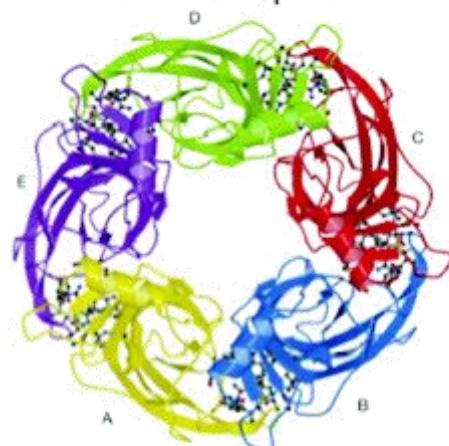
Unwin (2005) *JM Biol* 346:967



- Subunit composition is $(\alpha 1 \gamma)(\alpha 1 \delta)\beta 1$.
- Two ACh binding sites at $\alpha 1/\gamma$ and $\alpha 1/\delta$ interfaces regulate channel opening.
- There are 4 transmembrane domains in each subunit. M2 from each lines the channel.
- Most of the >100 amino acids in each large cytoplasmic domain pack too irregularly to be visualized in crystals.

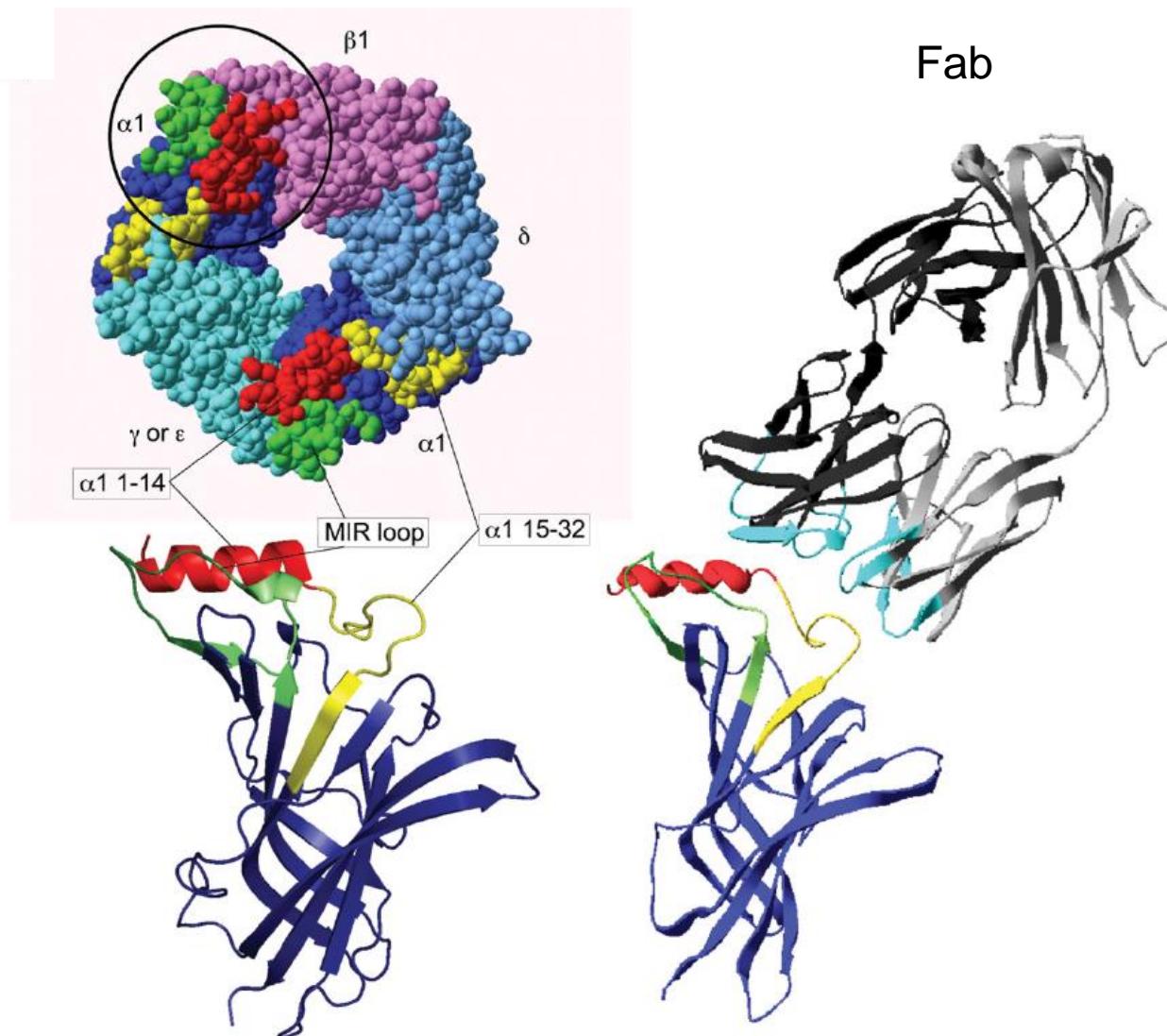
Crystal Structure of an ACh-binding Protein Reveals the Structure of AChR Extracellular Domains

Brejc et al. *Nature* (2001) 411:269



- AChBPs are water soluble proteins secreted by mollusk glia to modify cholinergic transmission. They are not found in vertebrates.
- There are 5 identical subunits.
- AChBPs crystallize easily because they lack transmembrane domains.
- AChBPs closely resemble the extracellular domain of $\alpha 7$ AChRs, but are a basic model for the extracellular domains of all AChRs and their cousins.

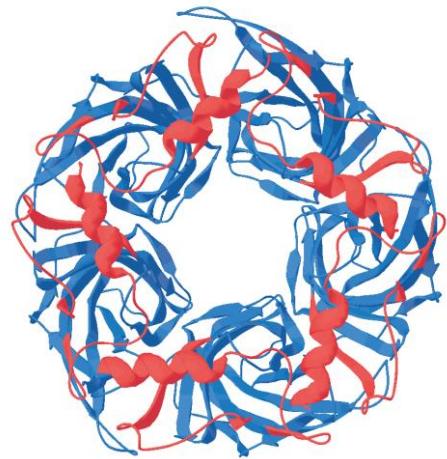
Model of the MIR Chimera and Its Interaction with an Antibody to the MIR Based on the Crystal Structure of AChBP and an IgG Fab



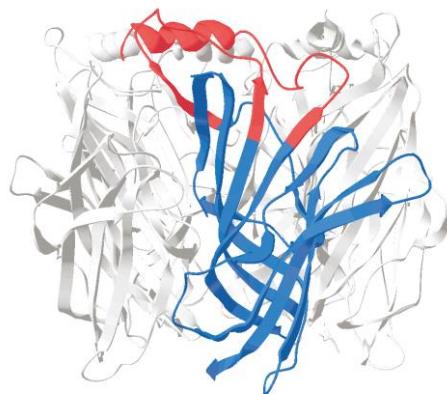
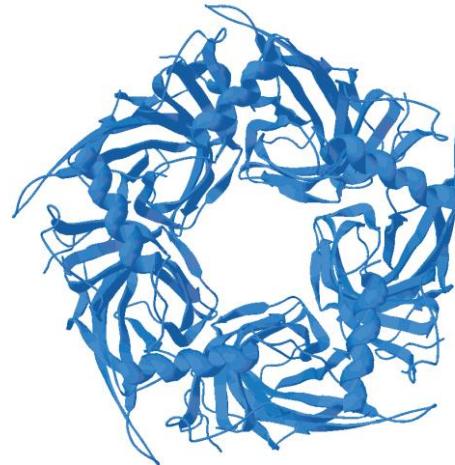
MIR (α 1-30, 60-81) AChBP Chimera Crystal Structure

Todd Talley et al., PDB ID:4ZJS

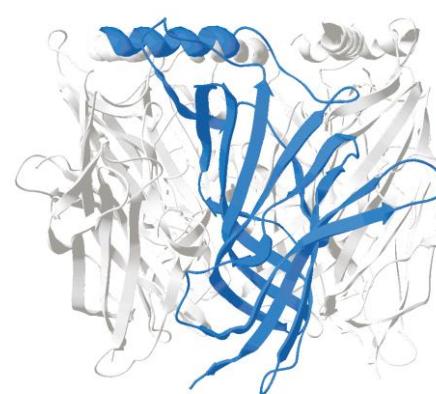
MIR/AChBP Chimera



Wild Type AChBP



red = α 1 1-30, 60-81



blue = AChBP

Immunization With AChR Impairs Neuromuscular Transmission As In MG

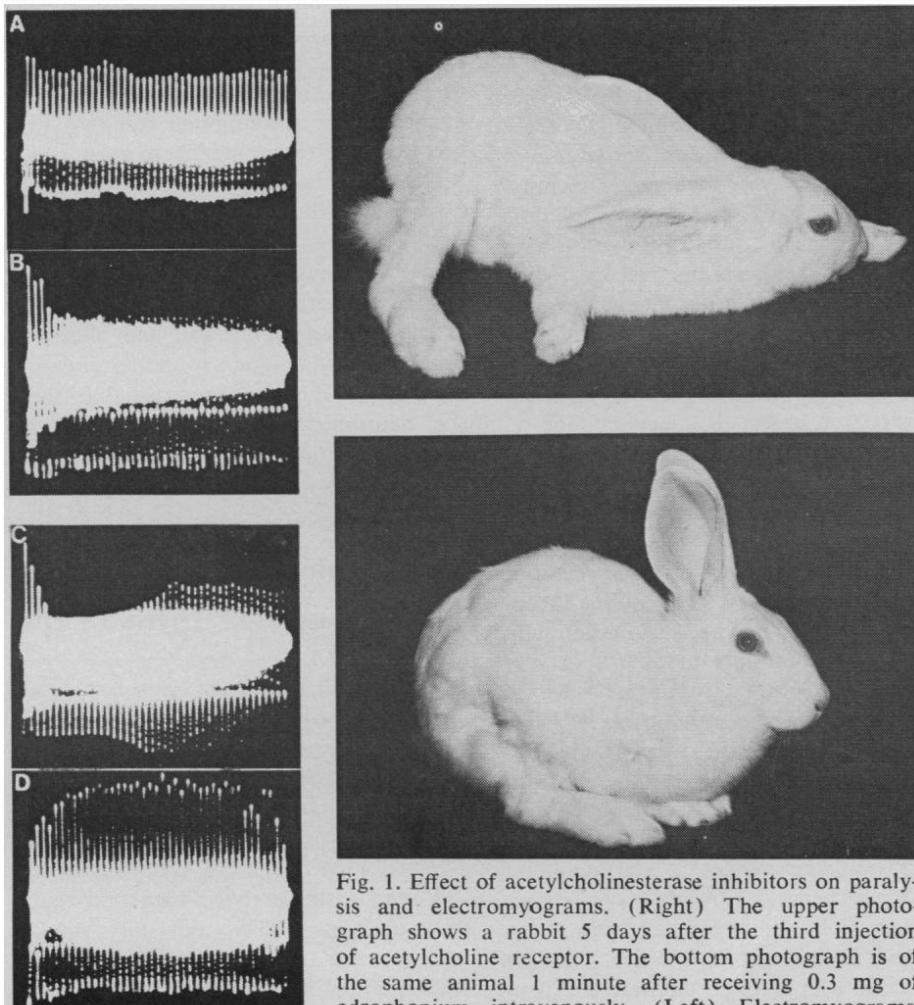
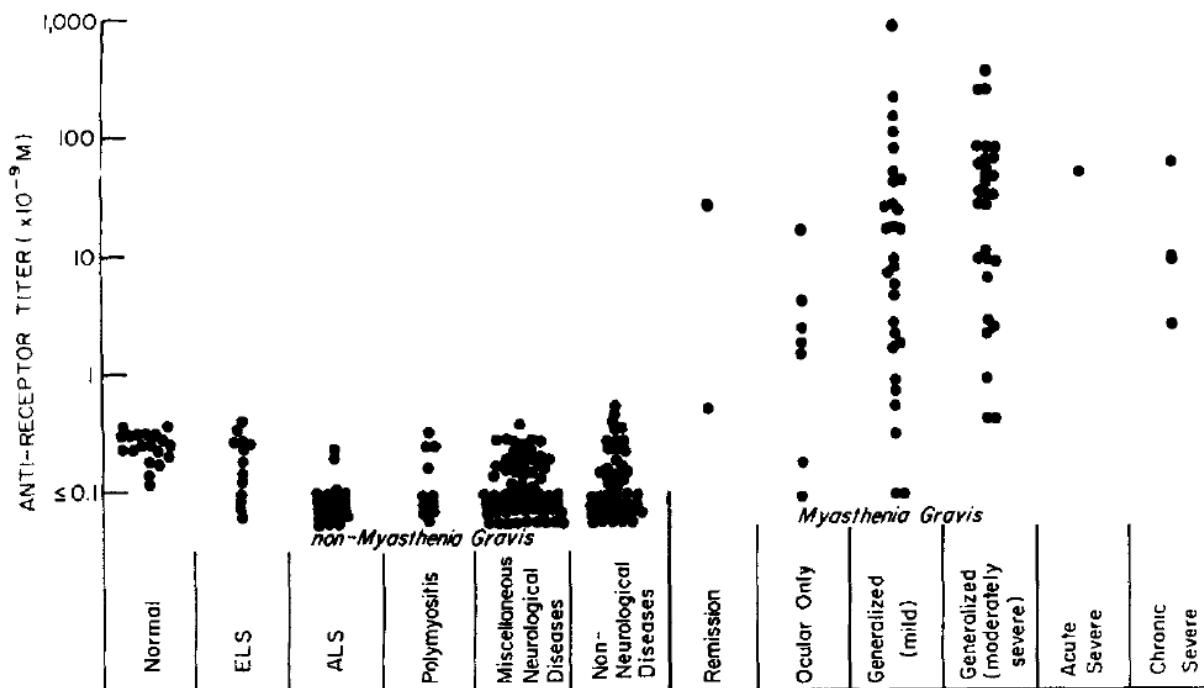


Fig. 1. Effect of acetylcholinesterase inhibitors on paralysis and electromyograms. (Right) The upper photograph shows a rabbit 5 days after the third injection of acetylcholine receptor. The bottom photograph is of the same animal 1 minute after receiving 0.3 mg of edrophonium intravenously. (Left) Electromyograms, before and after neostigmine injection, were taken on a control rabbit and on the rabbit seen on the right. Records were taken from a rear leg at a stimulus frequency of 40 per second. (A) Control before neostigmine; (B) experimental animal before neostigmine; (C) control after neostigmine; (D) experimental animal after neostigmine.

- J A Simpson (1960) Scott Med J 5:419 had suggested that MG was caused by autoantibodies to AChRs acting as competitive antagonists. Antibodies to AChR rarely act as competitive antagonists.

Most MG Patients Have Autoantibodies to Muscle AChRs



Lindstrom et al. (1976) Neurology 26:1054

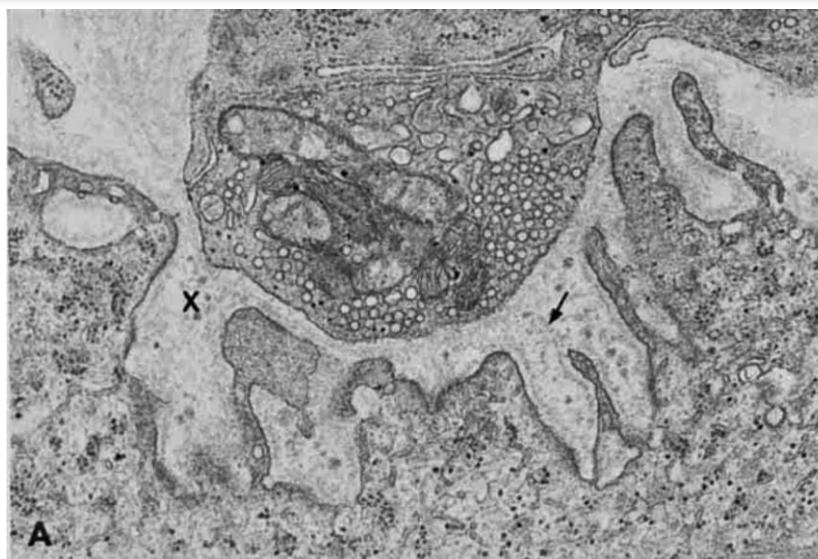
- The presence of autoantibodies to muscle AChR is immunodiagnostic for anti-AChR MG.
- Absolute antibody concentration does not closely correlate with severity, though ocular MG patients have lower titers and patients with thymoma have higher titers.
- Changes in antibody concentration correlate with changes in severity.

**Neuromuscular Junctions Have Folded Postsynaptic Membranes
With Semi-Crystalline Arrays of AChRs at the Tips of the Folds
Which Are Located Adjacent to Active Zones of ACh Release**

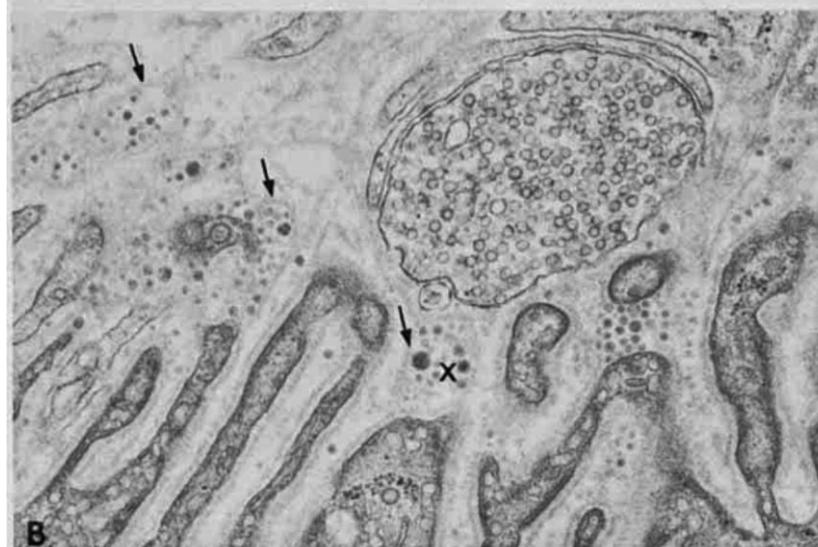


Engel et al. (1976) Ann N Y Acad Sci 274:60.

MG and EAMG Disrupt the Architecture of the Neuromuscular Junction



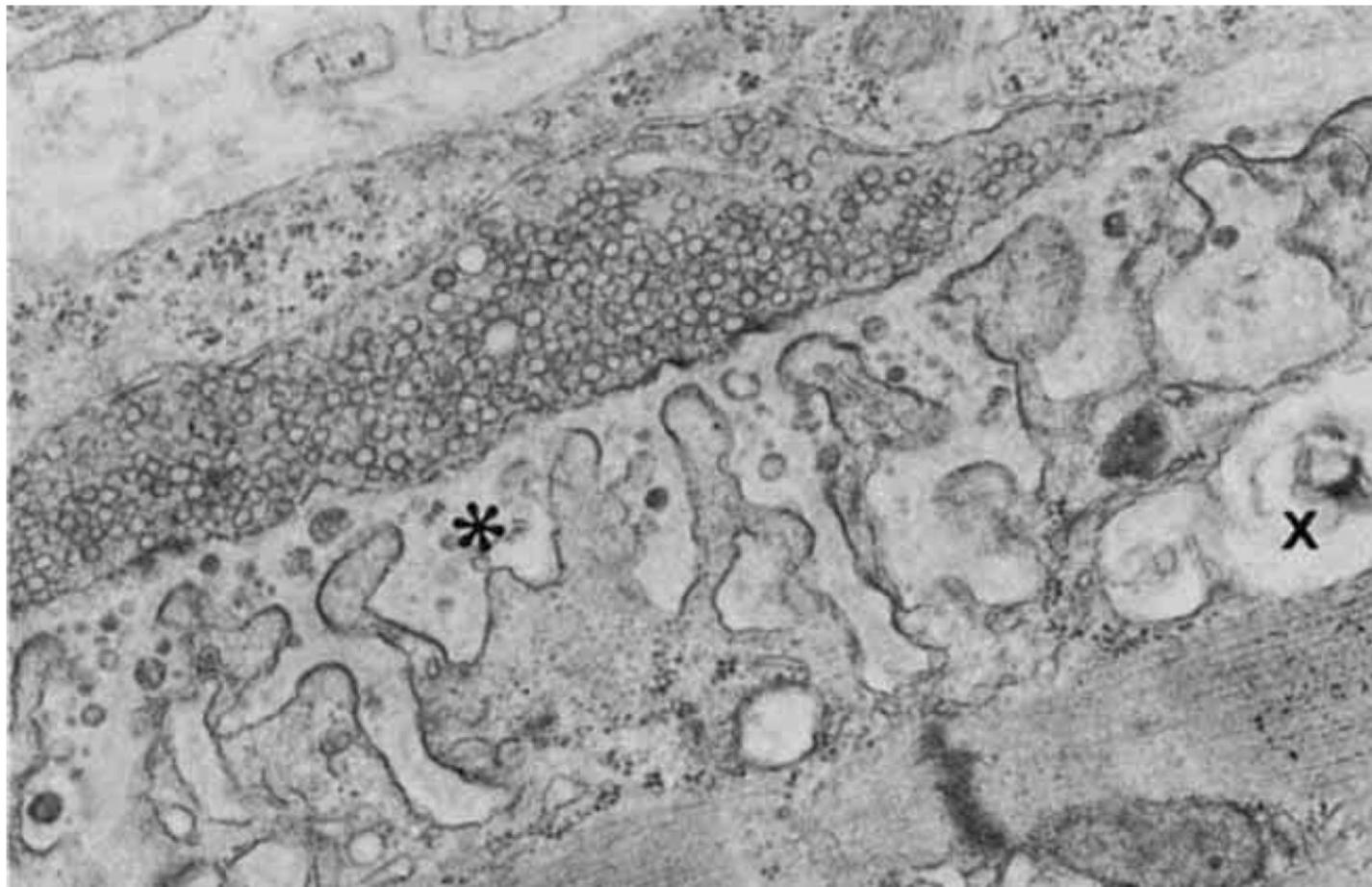
MG Endplate



EAMG Endplate

- Note degenerate remnants of the folds in the intersynaptic space (x). These can be labeled for AChR with cobra toxin-peroxidase and labeled for bound antibody and complement.

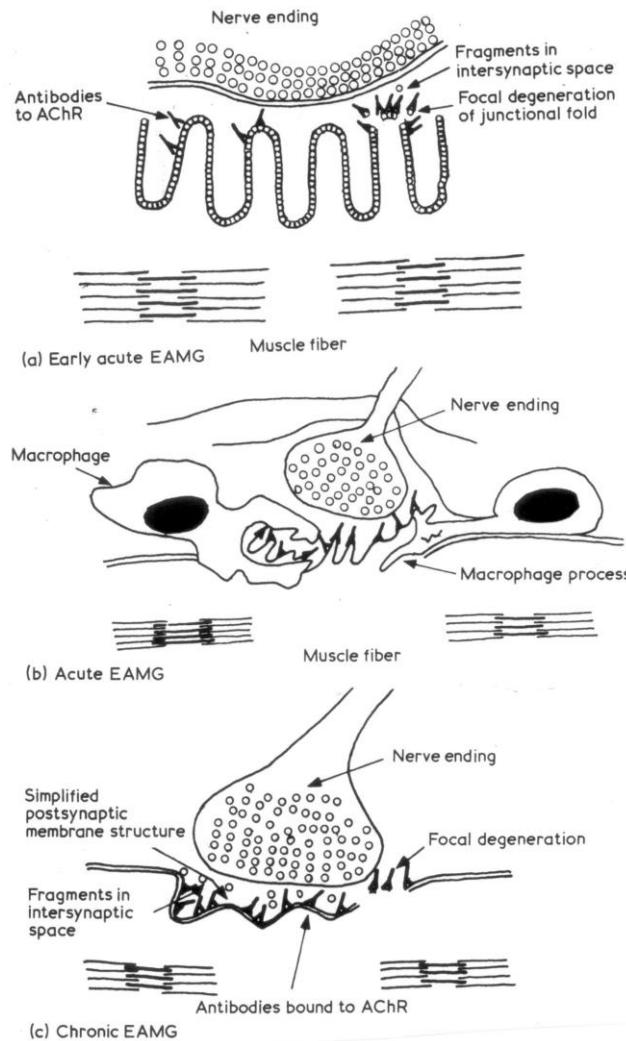
Note Degraded Postsynaptic Material in the Intersynaptic Space



Engel et al. (1976) Ann N Y Acad Sci 274:60.

- AChR shed in the immune assault may provide antigen to sustain EAMG and MG. This process may be the target of our specific immunosuppressive therapy.

Sudden Increase in Autoantibody Caused by Passive Transfer of Autoantibody or Active Immunization in Strong Adjuvants Causes a Transient Acute Phase of EAMG with Macrophages in Endplates Attracted by Release of C3 Complement Fragments. Macrophages are Not Seen in Chronic EAMG or MG.

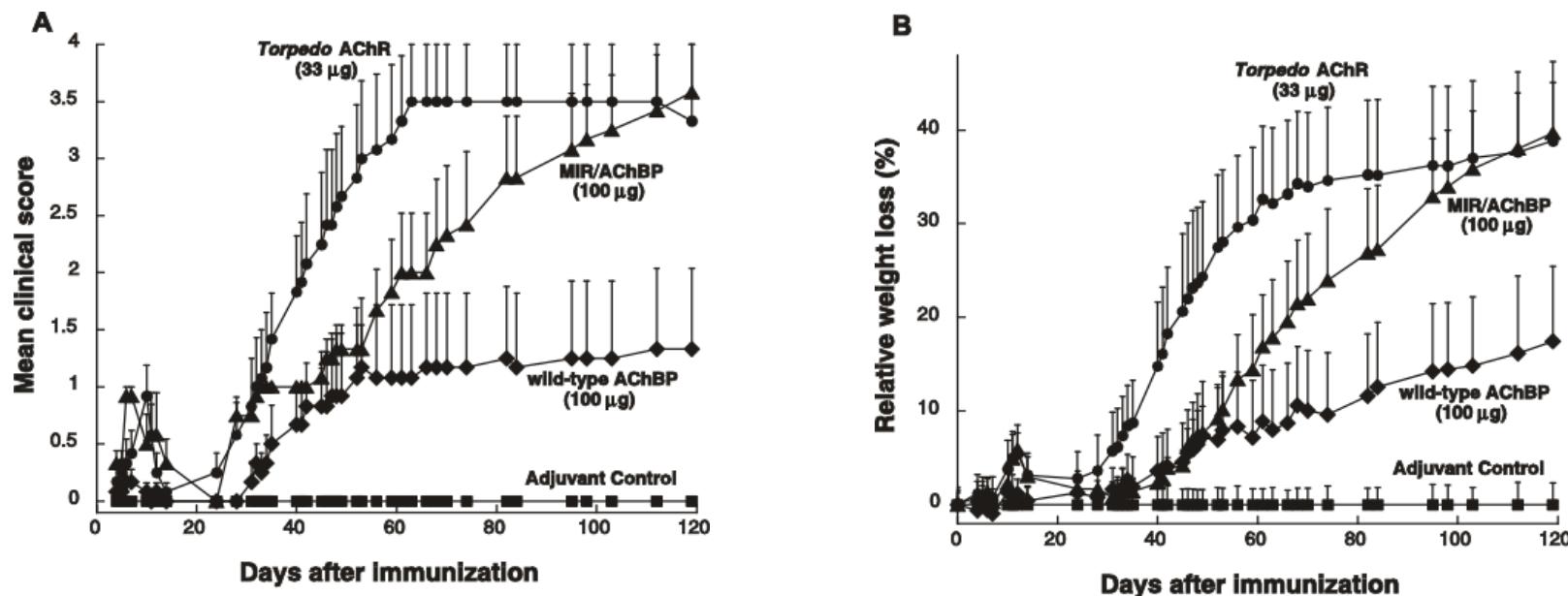


Lindstrom (1977) Receptors and Recognition. Series A, Volume 3:3.

History of Specific Immunosuppression of EAMG

- Reduced and carboxymethylated 6M guanidine denatured Torpedo AChR did not cause EAMG in rabbits, and prevented EAMG after subsequent immunization with native AChR. After induction of EAMG immunization with denatured AChR in CFA protected half of the rabbits.
Bartfeld and Fuchs (1978) PNAS 75:4006.
- Oral and nasal administration of native Torpedo AChR prevents onset of EAMG in rats. Suppression of ongoing EAMG is more difficult.
Wang et al. (1993) J Neuroimmunol 44:209; Okumura et al. (1994) Ann Neurol 36:704; Ma et al. (1995) J Neuroimmunol 58:51; Shi et al. (1998) Clin Exp Immunol 11:506.
- Nasal or oral administration of recombinant human or rat $\alpha 1$ extracellular domain prior to immunization with Torpedo AChR prevents EAMG, and given later suppresses EAMG.
Im et al. (2000) J Neuroimmunol 111:161; Maiti et al. (2004) J Neuroimmunol 152:112.
- However, a more native $\alpha 1$ extracellular domain exacerbates EAMG. Removing the MIR and another epitope restored a therapeutic effect.
Im et al. (2000) J Immunol 165:3599; Yi et al. (2008) Mol Immunol 46:192.
- Thus, AChR-specific immunosuppressive therapy is possible in principle, but these results do not provide a foundation for translation to therapy for MG.

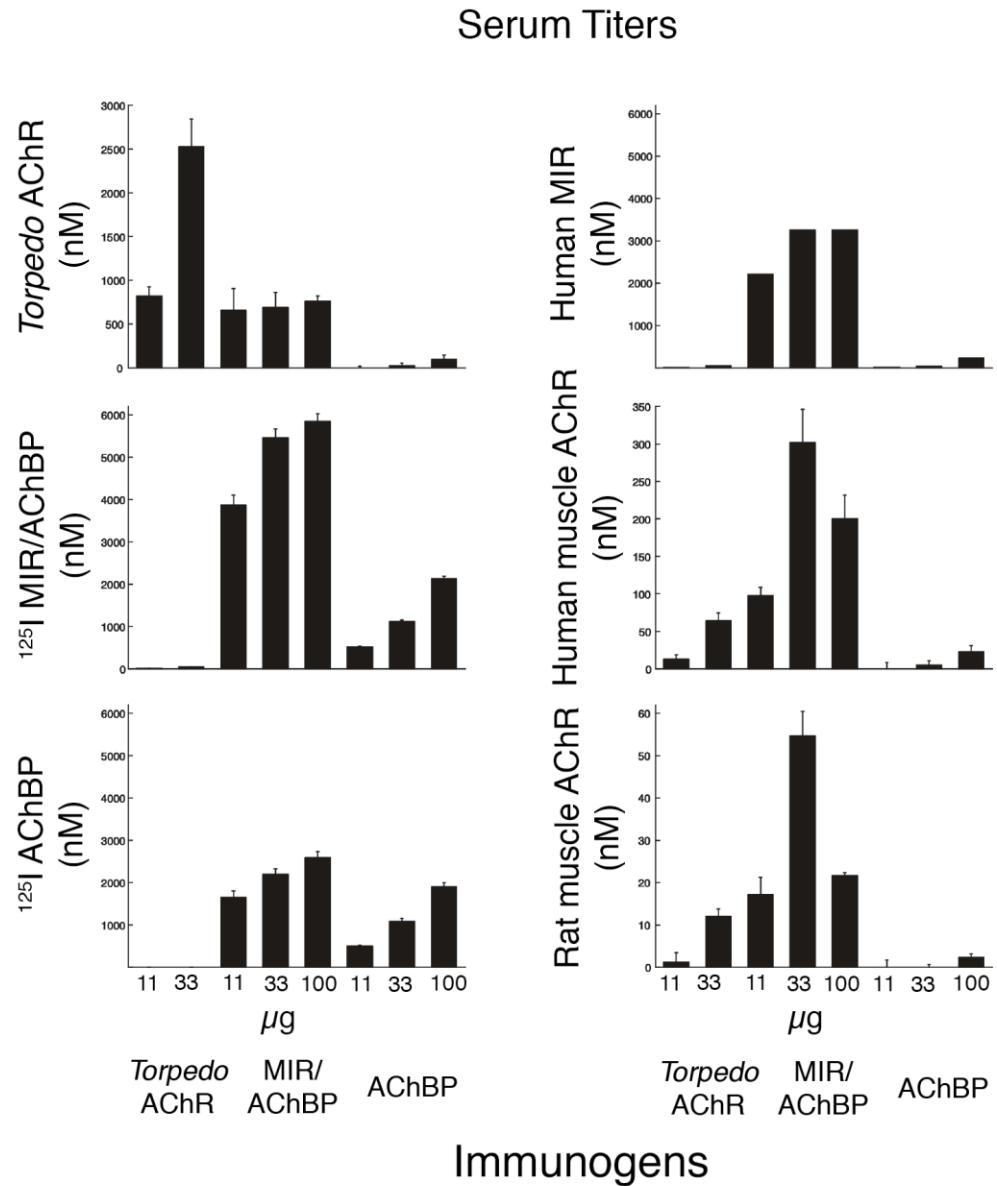
Comparison of Induction of EAMG Using AChR, MIR/AChBP α 1 (1-30, 60-81) Chimera, or Wild Type AChBP



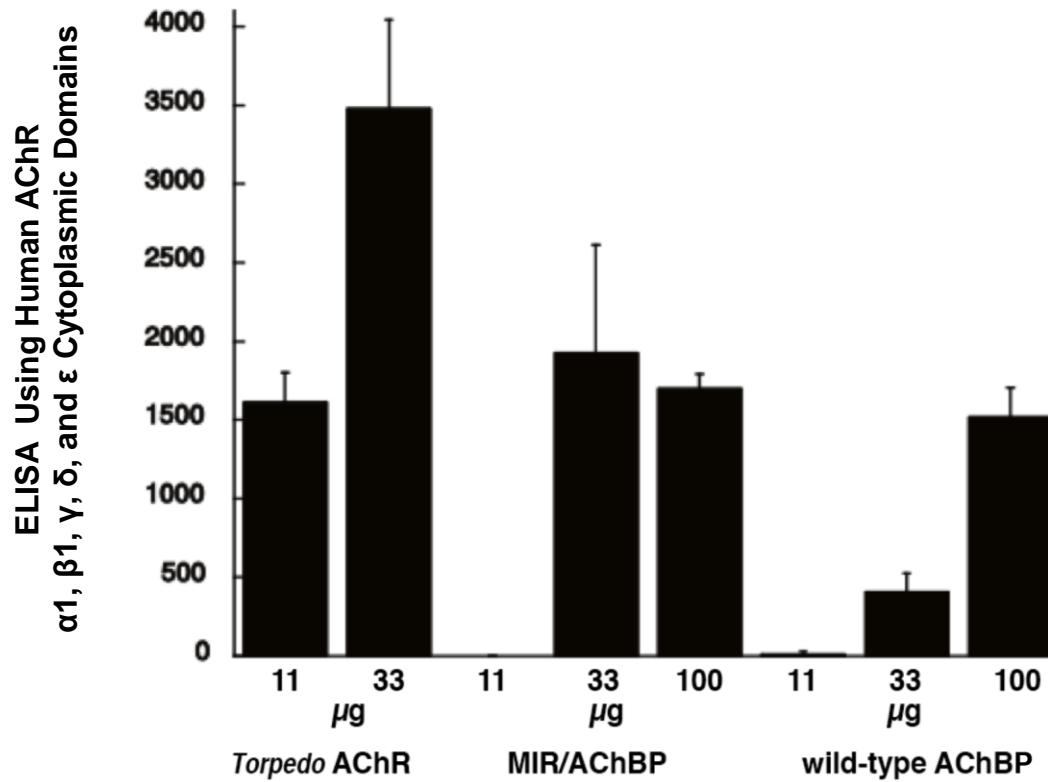
- Weight is a good quantitative proxy for clinical score.
- MIR/AChBP efficiently induces both acute and chronic EAMG with $\leq 1/3$ the potency of intact AChR and full efficacy (6/6 rats sick).
- Surprisingly, wild type AChBP could induce chronic EAMG, though not acute, slowly and with low potency and efficacy (3/6 rats sick).
- Thus, MG might be induced by crossreaction with some AChR-like protein, perhaps of microbial origin.

Immunogenicity

- Torpedo AChR, human $\alpha 1$ MIR/AChBP and AChBP induce primarily species-specific antibodies.
- The $\alpha 1$ MIR is especially immunogenic.
- < 1% cross-reaction with rat $\alpha 1^*$ AChR is sufficient to induce EAMG.
- Rats try hard to avoid an autoimmune response to the MIR: the MIRs of rats and human differ by 4 amino acids, but rats make 6 fold more antibodies species-specific for human MIR.

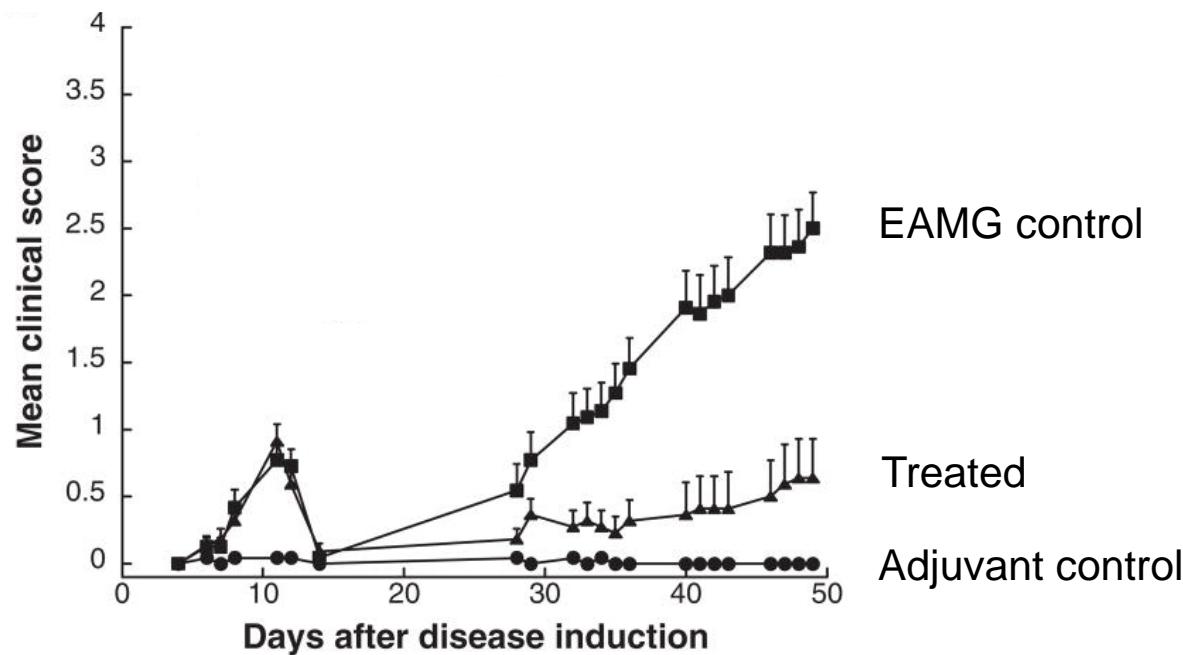


Rats That Develop EAMG Develop High Titers of Antibodies to Cytoplasmic Domains of Muscle AChR, Even When the Immunogen Does Not Contain Cytoplasmic Domains (i.e. MIR/AChBP and AChBP)



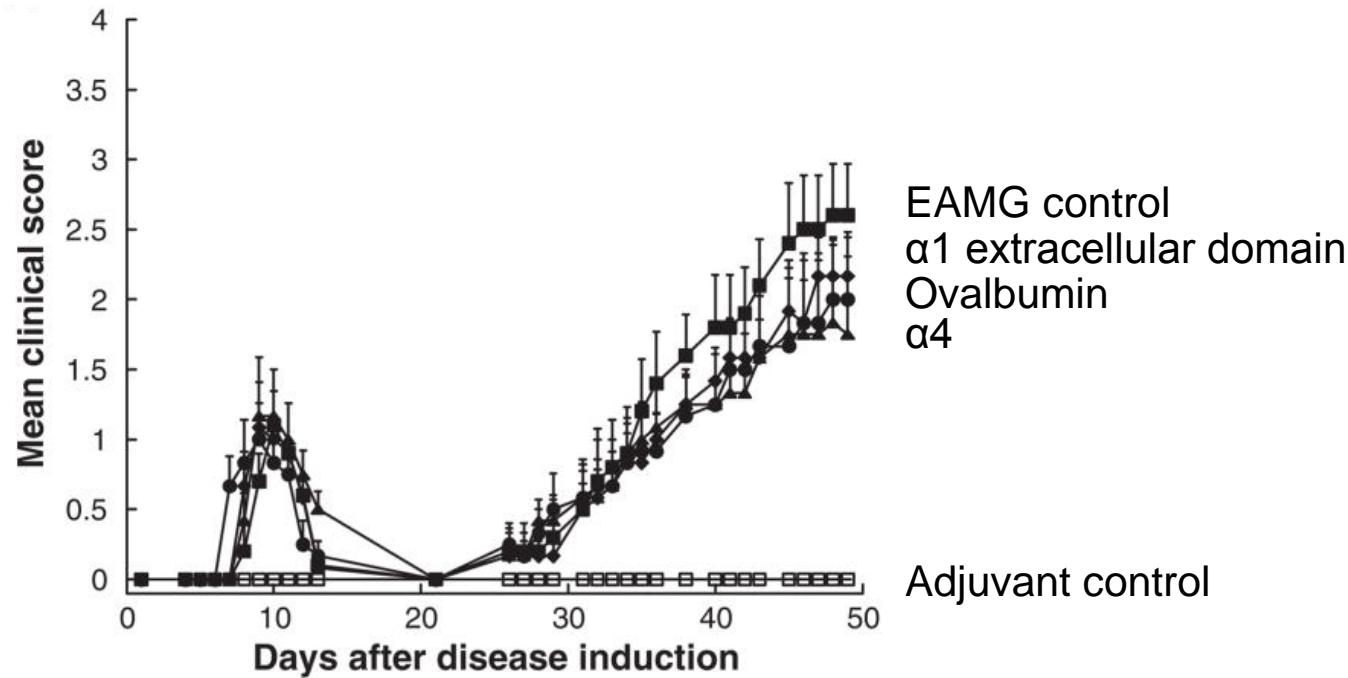
- Immunization with the extracellular domain of the AChR disrupts muscle AChRs revealing cytoplasmic epitopes that stimulate an autoimmune response.
- The initial immunization stimulates a self-sustaining autoimmune response to muscle AChR.

EAMG Can Be Suppressed by i.p. Administration of a Mixture of Bacterially-Expressed Human Muscle AChR Constructs Containing the Extracellular and Cytoplasmic Domains of $\alpha 1$, $\beta 1$, γ , δ and ϵ Subunits



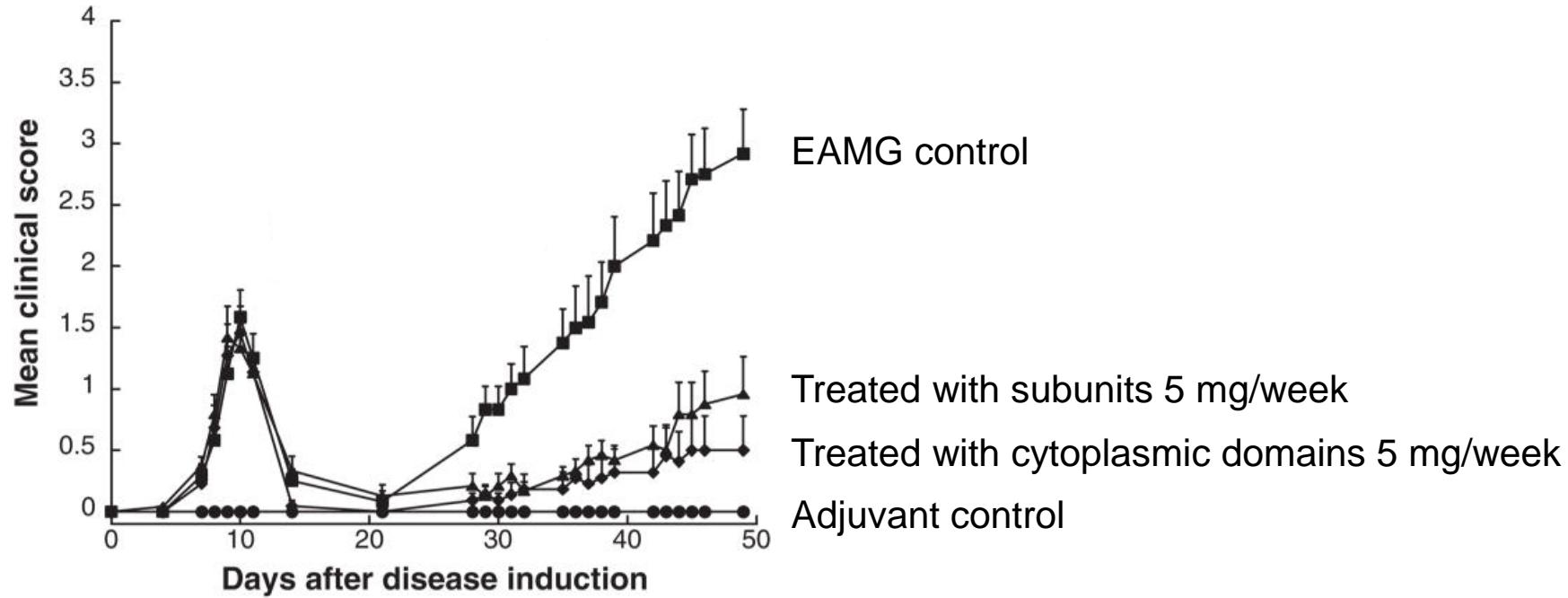
- EAMG was initiated with 35 µg Torpedo AChR in TiterMax adjuvant at day 0.
- Therapy started at day 14, right after the acute phase, 5 mg i.p. each week.

EAMG Was Not Suppressed by i.p. Administration of 5 mg/Week Doses of Ovalbumin, or Bacterially-Expressed Human AChR α 4 Extracellular and Cytoplasmic Domains, or α 1 Extracellular Domains



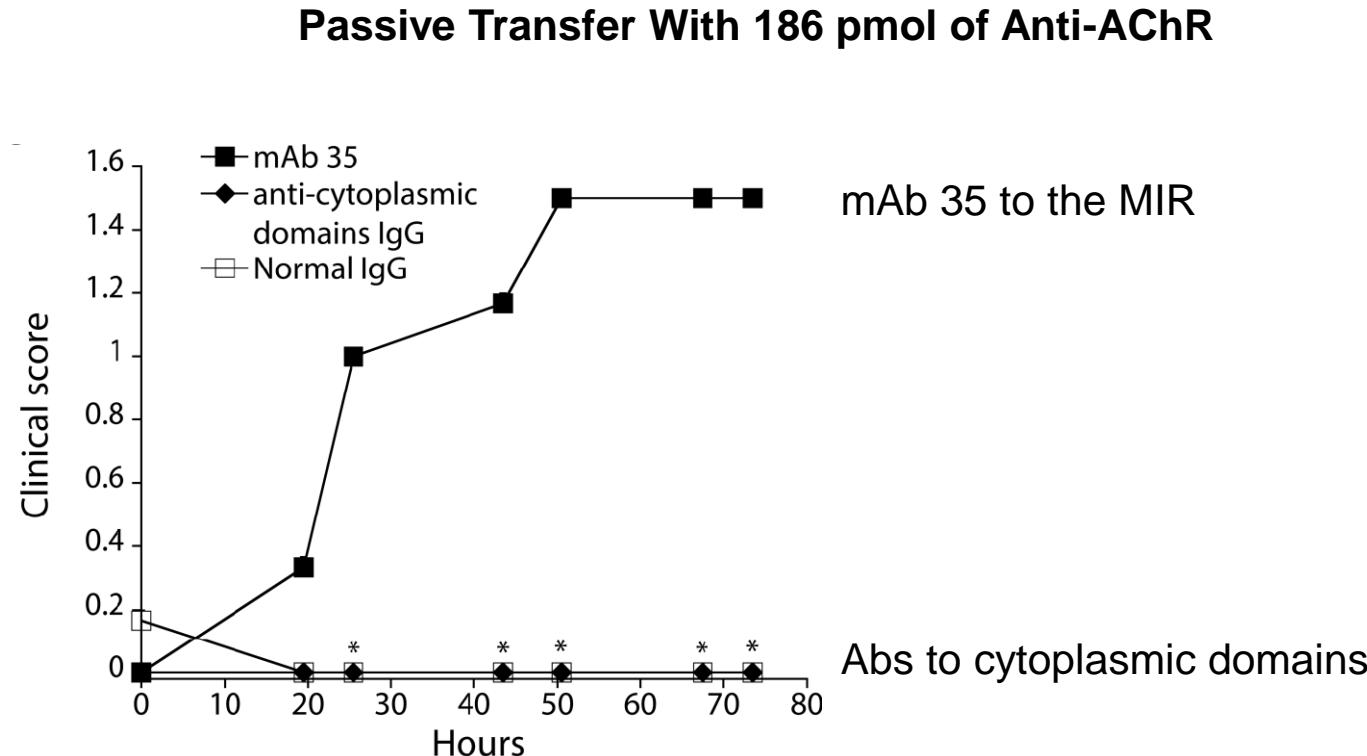
- Irrelevant antigens do not suppress EAMG.
- α 1 extracellular domain does not suppress EAMG because it can induce EAMG due to partial renaturation of the MIR.
- Presence of extracellular domains in a therapeutic construct limits its effectiveness, and risks promoting EAMG.

Treatment With Only AChR Subunit Cytoplasmic Domains Is More Effective Than Treating With Both Extracellular and Cytoplasmic Domains



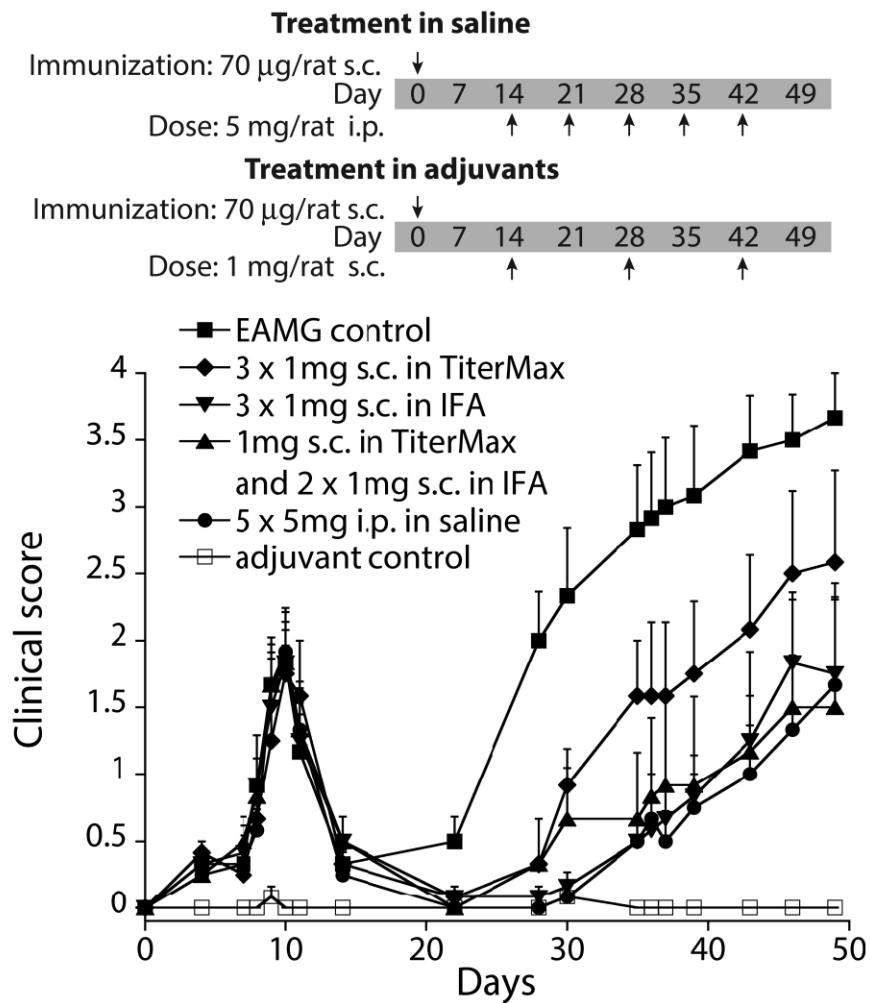
- Therapy with only cytoplasmic domains prevents exacerbating EAMG with extracellular antigens

Immunization With Cytoplasmic Domains (0.5 mg in TiterMax Adjuvant) Followed by 3 Boosts in IFA at 3 Week Intervals Produces A High Concentration of Antibodies to Rat Muscle AChR (243 nM), But No EAMG or Ability to Passively Transfer EAMG



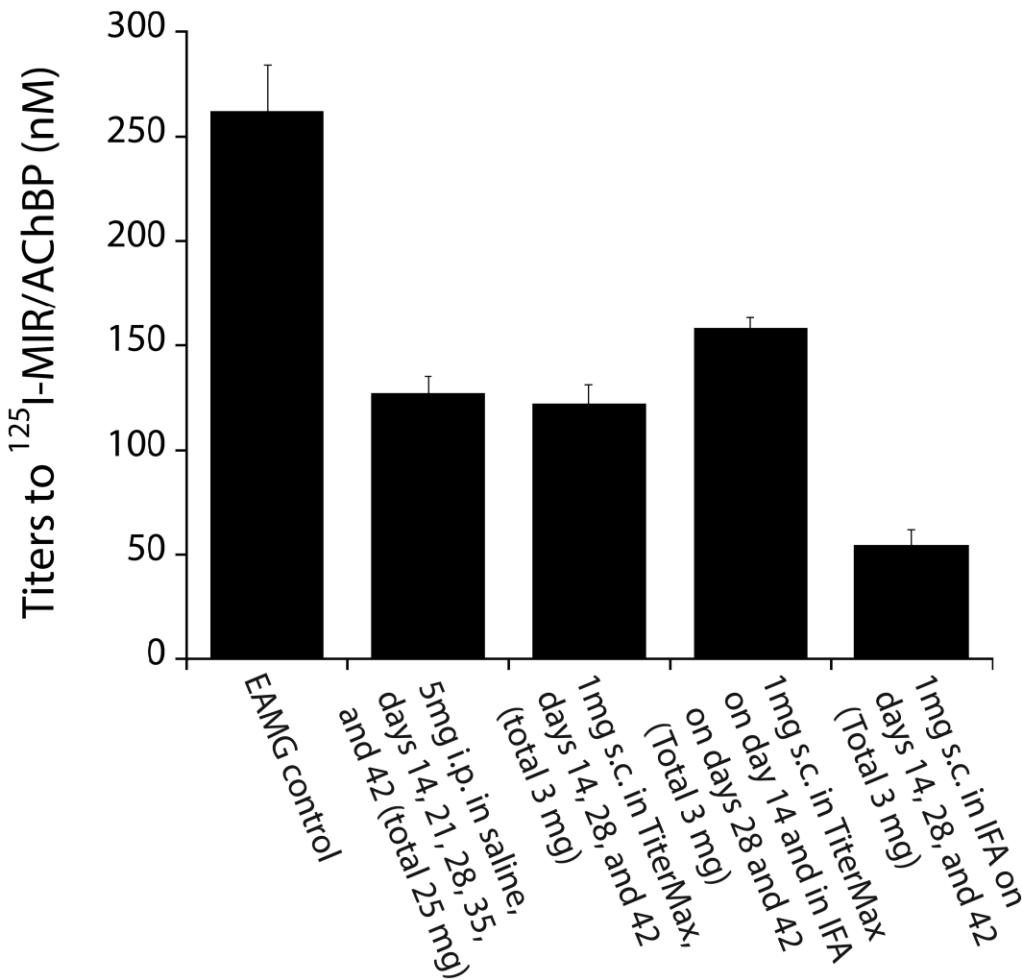
- Thus, this therapy is safe because it does not induce a pathological autoimmune response to AChRs

Therapeutic Immunization With Cytoplasmic Domains Is More Effective Using Adjuvant



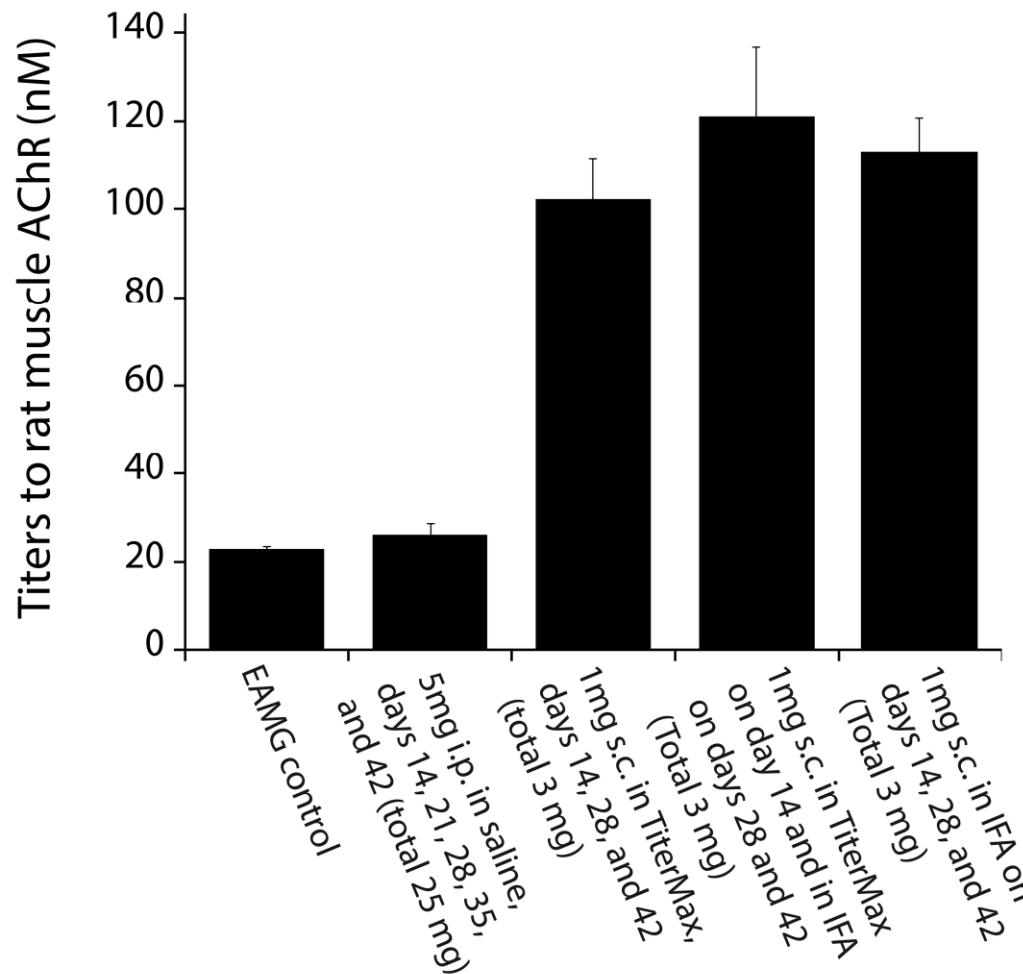
- s.c. IFA increases potency of cytoplasmic domains 8 fold compared to i.p. in saline.
- The weaker adjuvant Incomplete Freund's Adjuvant (IFA) is better than the stronger adjuvant TiterMax.

Therapy Decreases Pathological Autoantibodies to the MIR



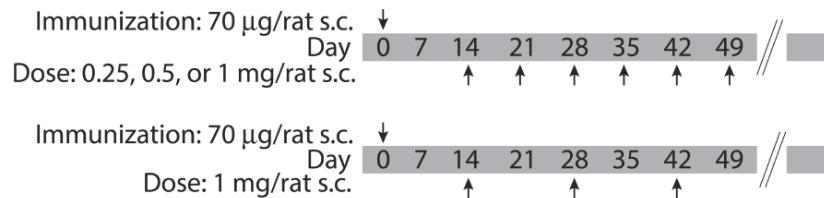
- Repeated low doses in IFA are most effective.

Therapy Increases Antibodies to Solubilized Muscle AChR As a Result of Antibodies to the Therapeutic Cytoplasmic Domains

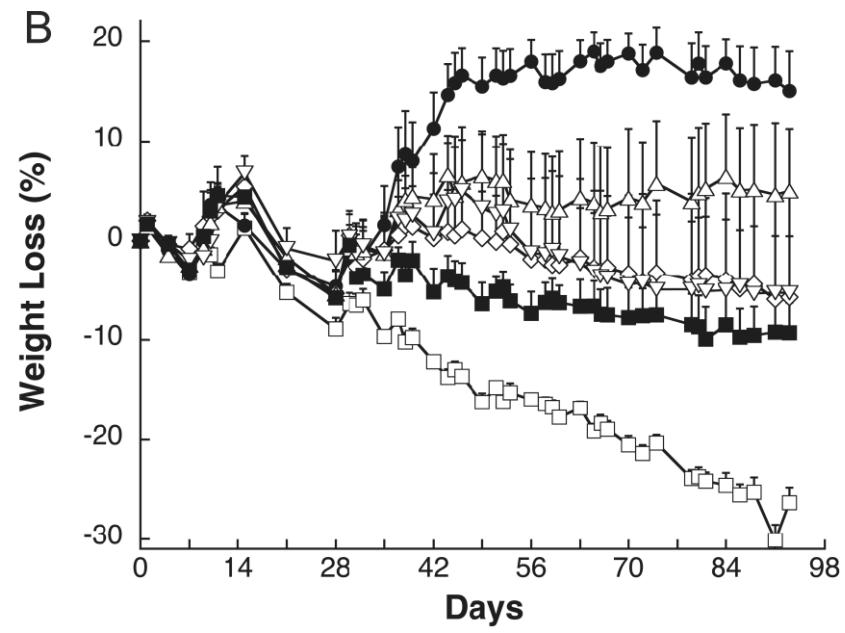
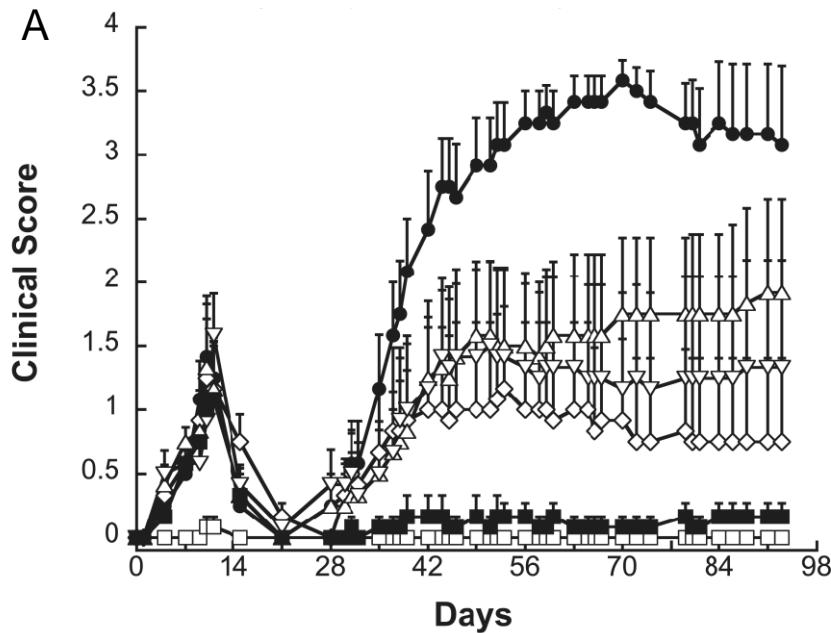


- Autoantibodies to the cytoplasmic domain can not bind to muscle AChRs in vivo

Optimizing Dose and Schedule of Therapeutic Immunization in IFA

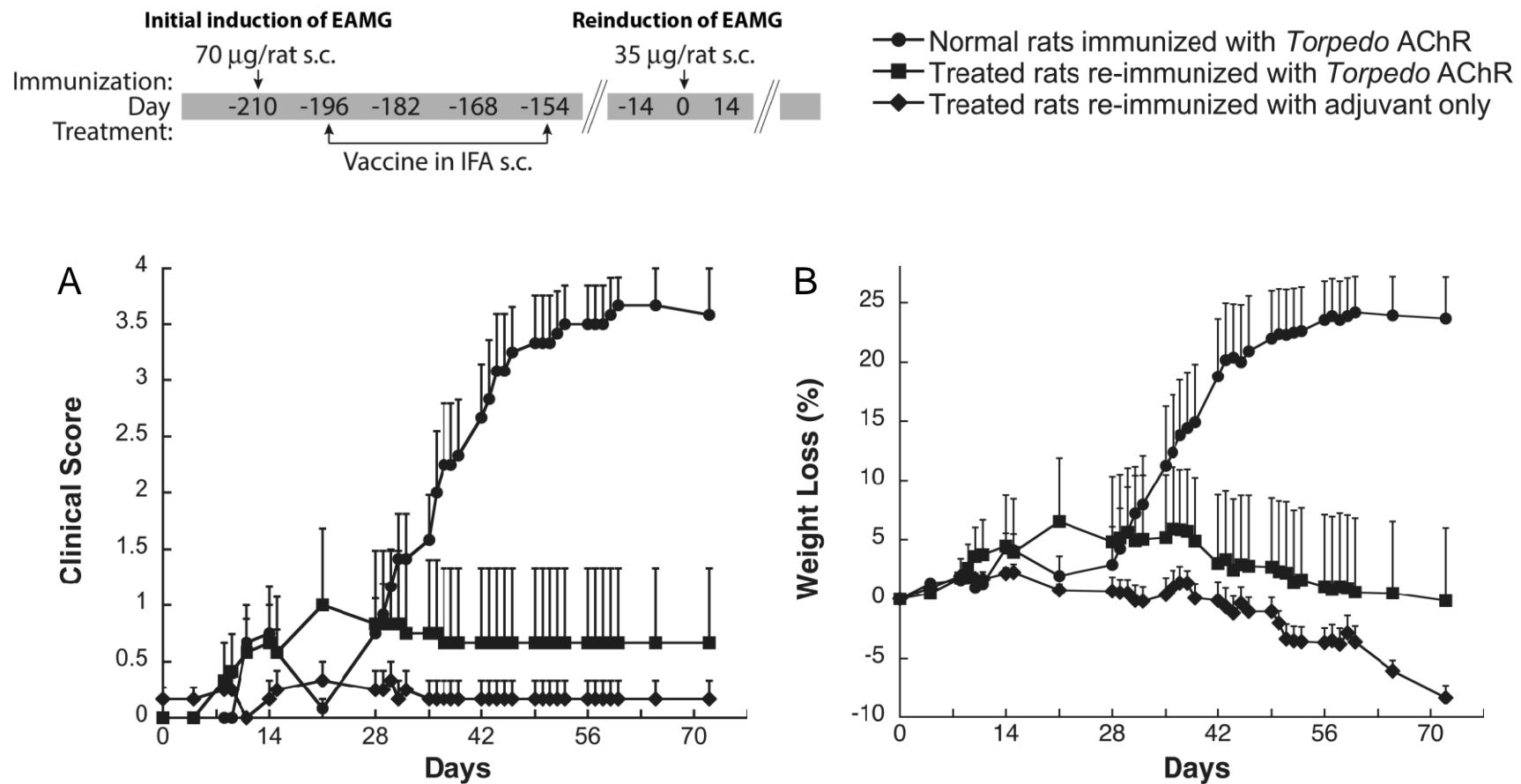


● EAMG control
△ 0.25 mg weekly
▽ 0.5 mg weekly
◇ 1 mg every two weeks
■ 1 mg weekly
□ Adjuvant control



- Six 1 mg doses at 1 week intervals prevent onset of chronic EAMG.

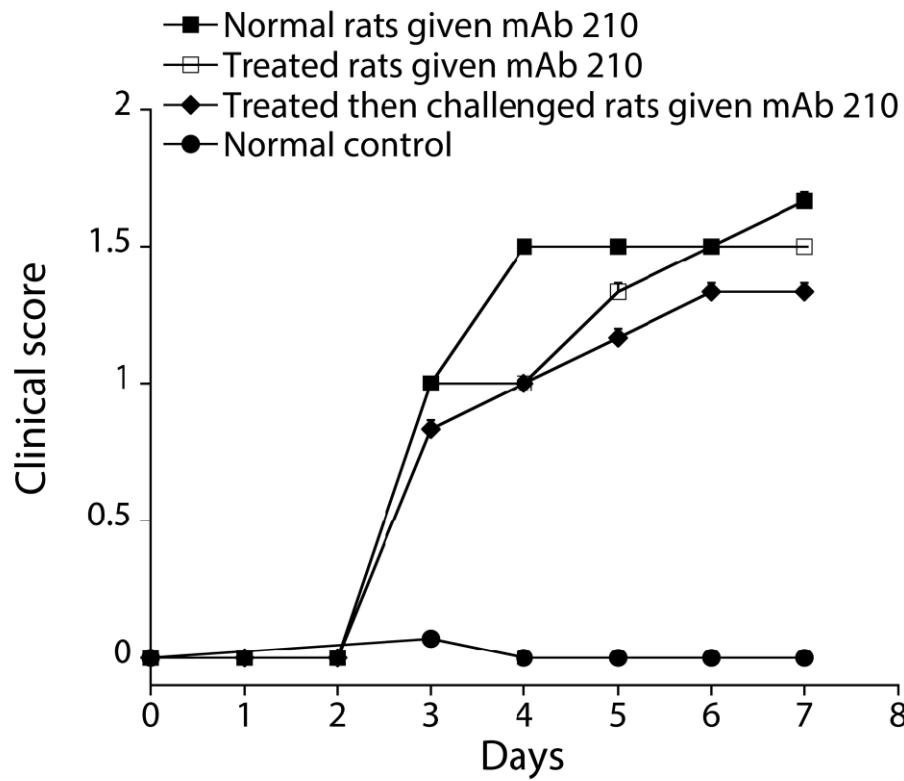
Successfully Treated Rats Are Resistant To Re-Induction of EAMG



- Rats successfully treated with 0.25 to 1 mg/week of cytoplasmic domains for 6 weeks were resistant to EAMG when immunized with *Torpedo AChR* 6 months later.
- Thus, after an effective short course of therapy MG patients might remain healthy for at least a long time, perhaps indefinitely.

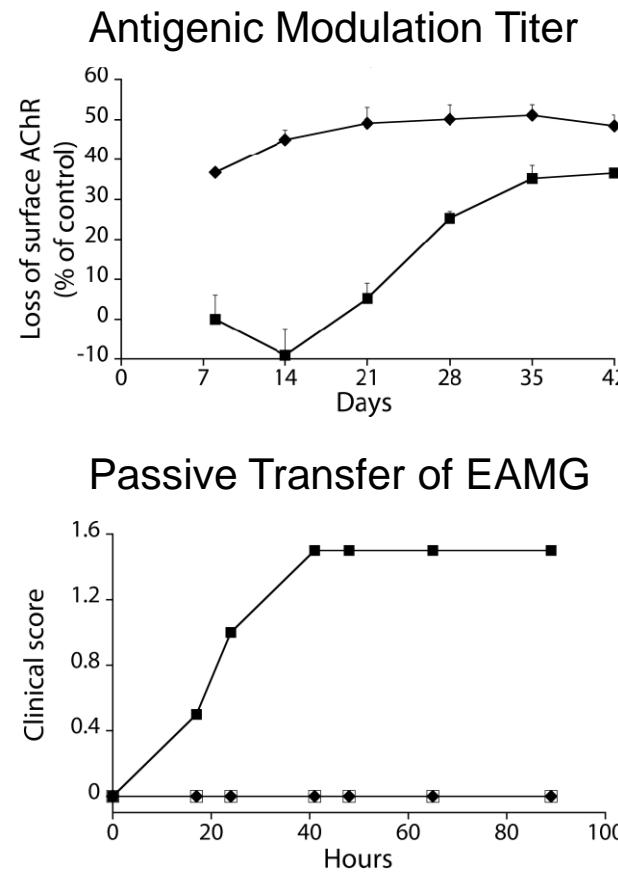
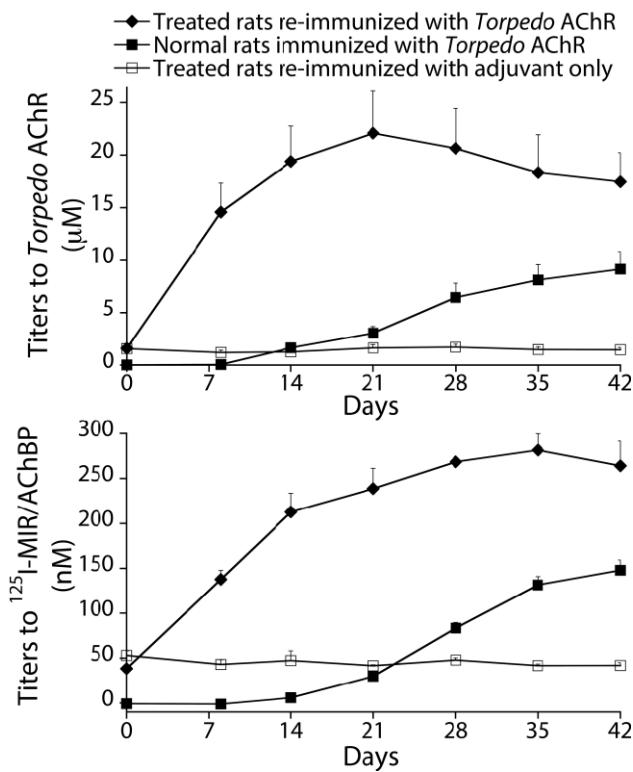
Resistance of Treated Rats to Re-Induction of EAMG Is Not Because Their Endplates Are Altered

Passive transfer of EAMG by mAb 210 to the MIR



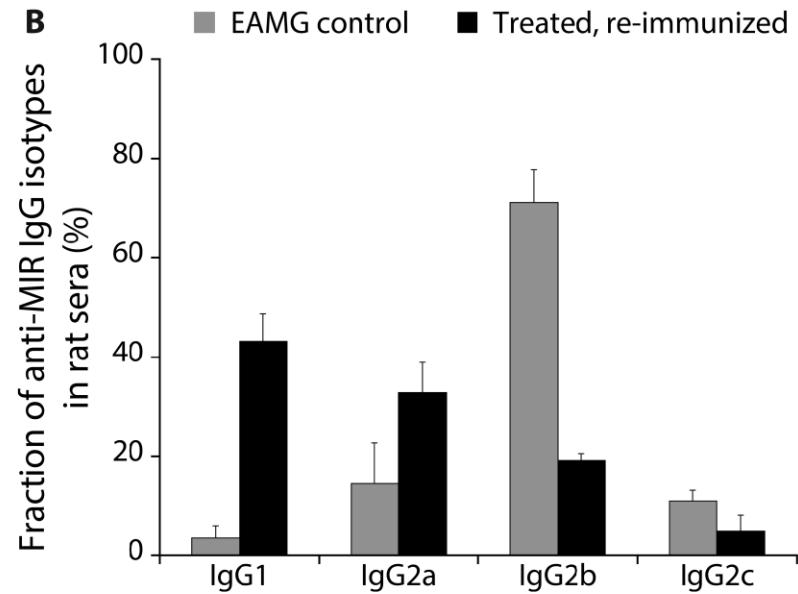
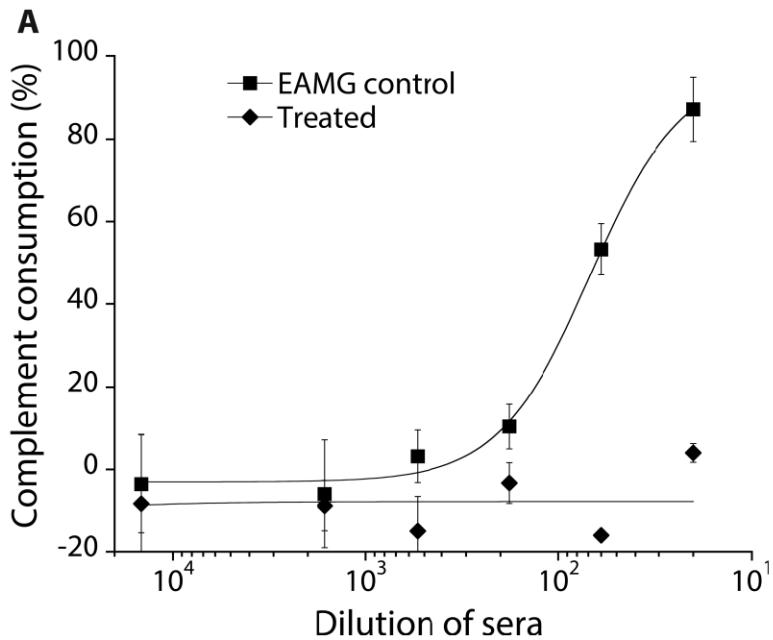
- Successfully treated rats are resistant to making new antibodies to AChR that cause EAMG, but they are susceptible to passive transfer of pathological EAMG antibodies.
- Their immune responses have changed, their neuromuscular junctions have not.

Re-Immunization of Treated Rats Rapidly Produces High Titers of Antibodies to Torpedo AChR and the MIR Effective at Antigenic Modulation But Not at Passive Transfer of EAMG



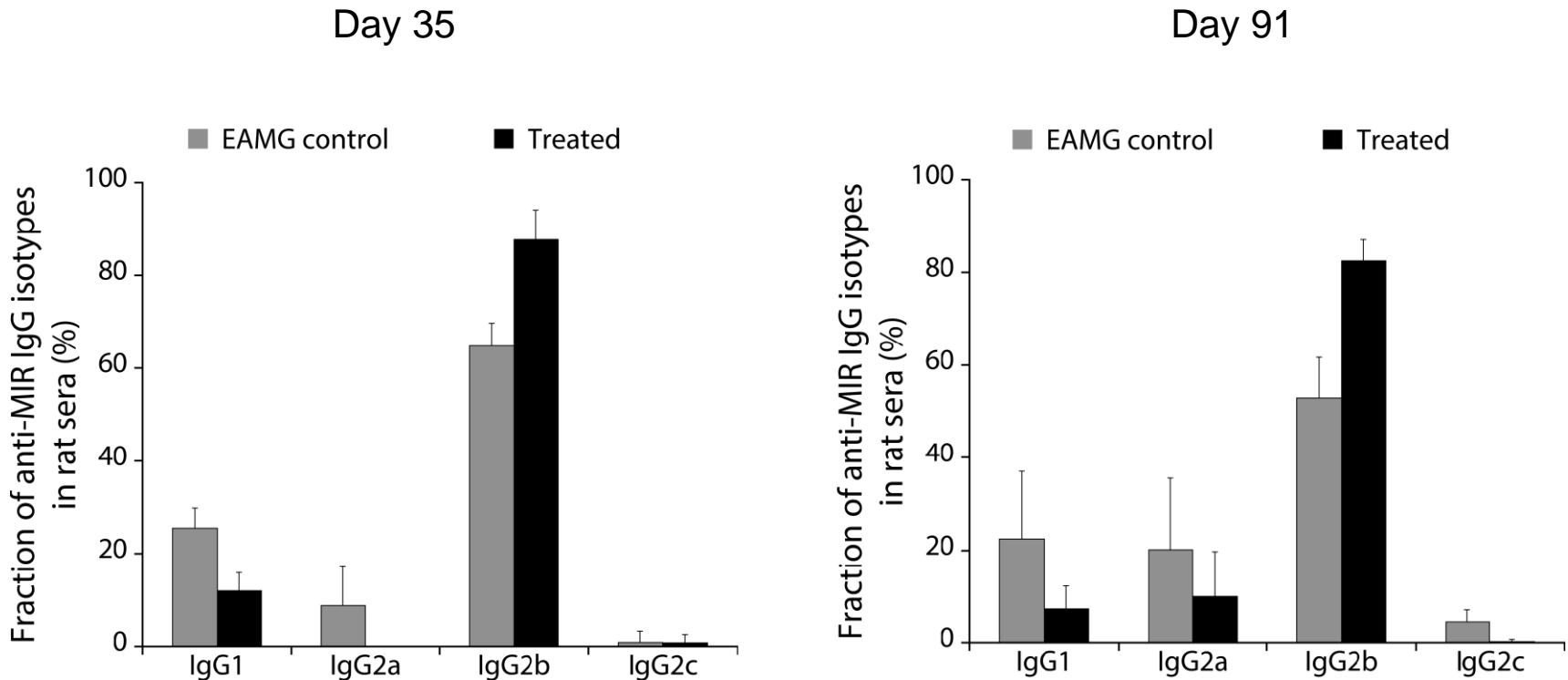
- Antigenic modulation depends on the ability of IgG to cross-link AChRs, thereby increasing their rate of internalization and destruction.
- Passive transfer depends on the ability of IgG to fix complement, thereby causing focal lysis and targeting the postsynaptic membrane for attack by macrophages.

Antibodies to the MIR From Treated Rats Re-Immunized With AChR Are Less Effective at Fixing Complement Because of Isotype Switching



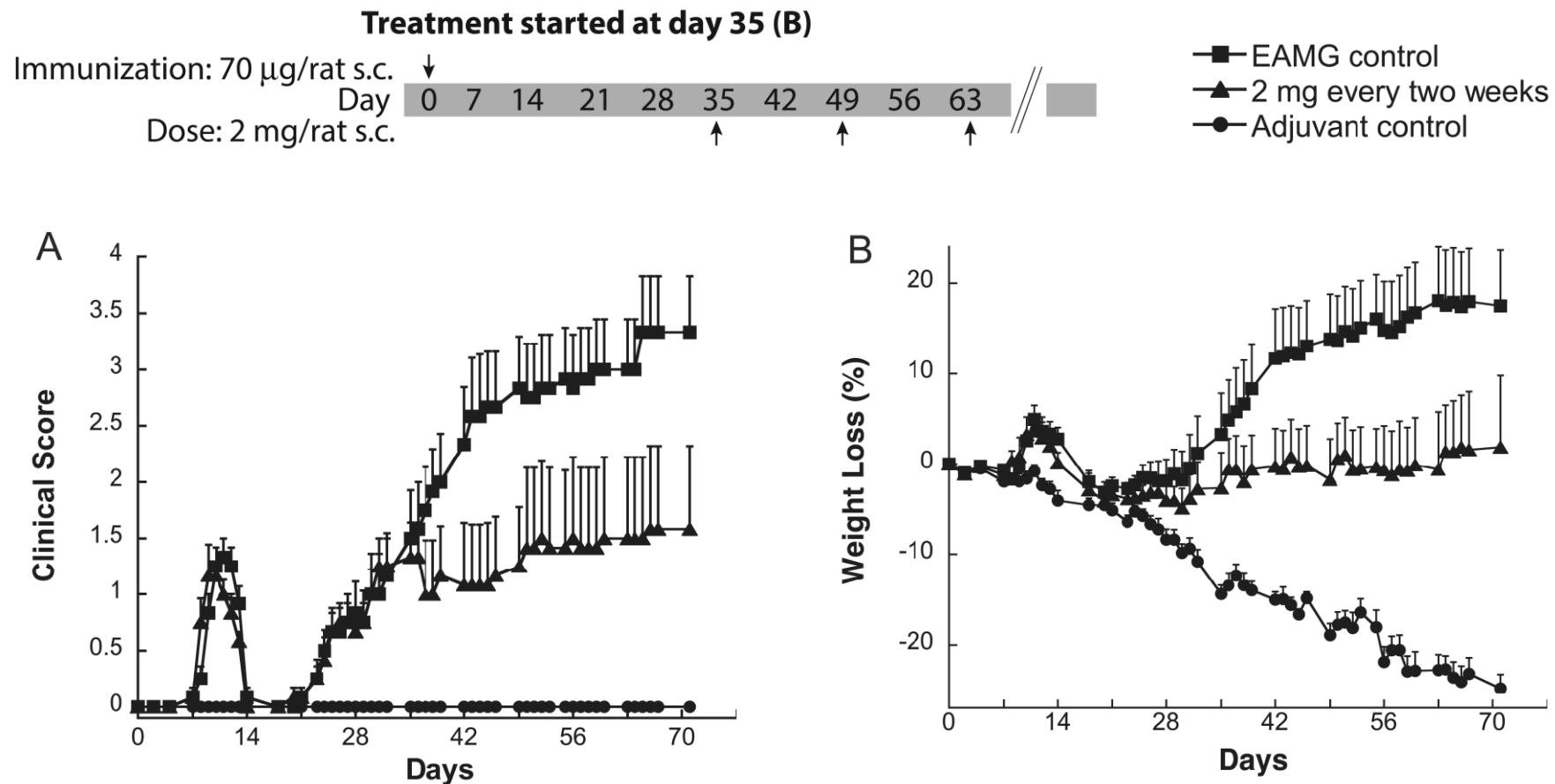
- IgG2b is most potent at fixing complement, IgG2a is less efficient, and IgG1 much less efficient.
- Successfully treated rats, if immunized again with Torpedo AChR, preferentially make antibodies to AChR of isotypes that do not fix complement, thus are not potent pathogens even though they can bind to the extracellular surface of muscle AChRs.

Successful Therapy With Cytoplasmic Domains Does Not Alter the Isotype of Antibodies to the MIR in the Short Term (Day 35) or the Long Term (Day 91).



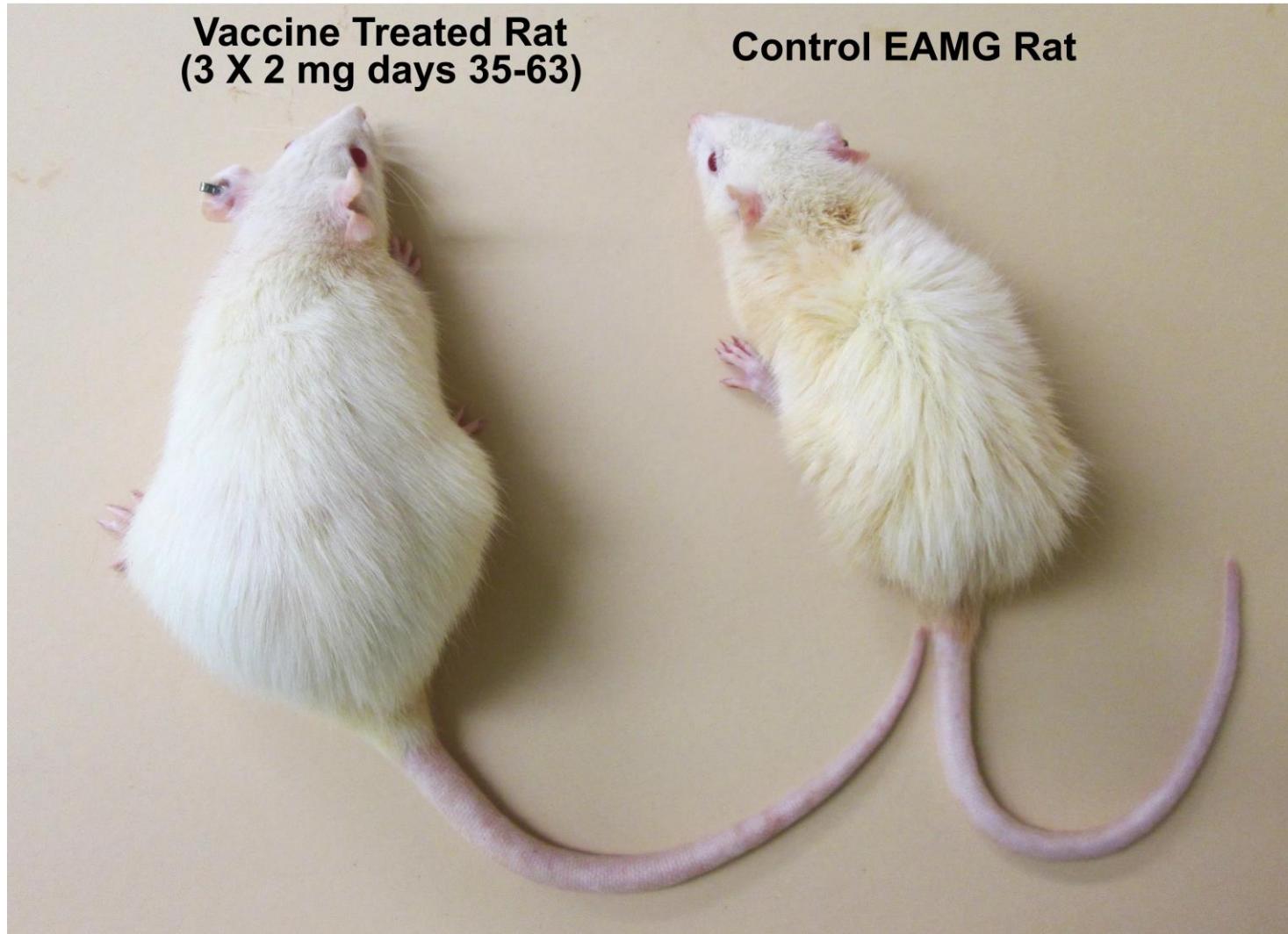
- Thus, therapeutic benefit does not depend on isotype switching. Isotype switching is a delayed response to re-immunization after therapy.

Treatment During the Chronic Phase Rapidly Suppresses Further Development of Chronic EAMG

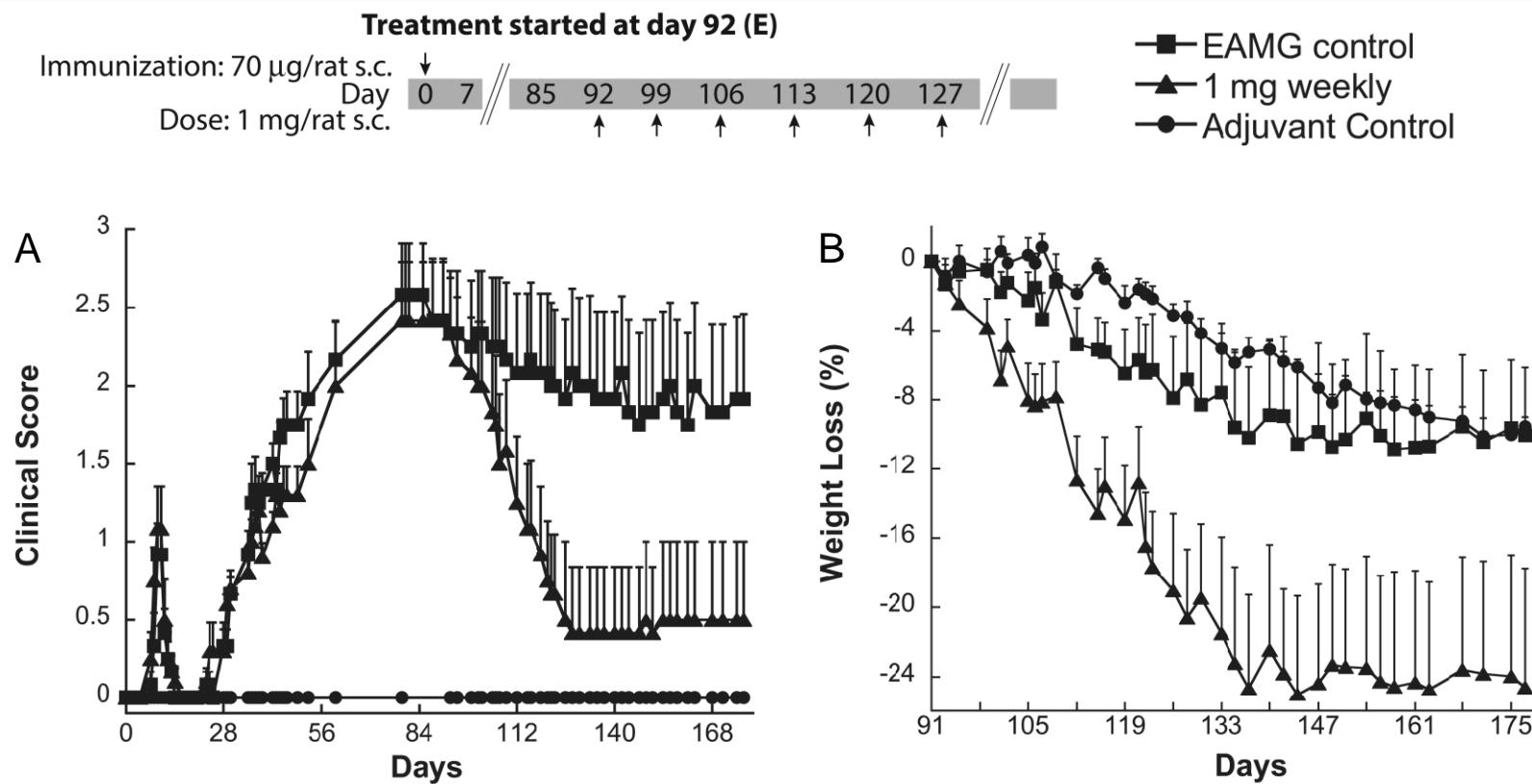


- 4/6 untreated died, 1/6 treated died and 3/6 returned to normal
- Non-specific immunosuppressive therapy of MG often is not effective for months, perhaps due to sustained production of pathological antibodies by plasma cells.

Specific Immunosuppressive Therapy With AChR Cytoplasmic Domains Relieves Weakness and Allows Treated Rats to Gain Weight



The Most Effective Dose and Schedule for Treatment During the Chronic Phase is 1 mg in IFA Each Week for 6 Weeks



- 21 rats were given EAMG to provide enough surviving EAMG rats to begin therapy at day 92.
- Treatment of ongoing chronic EAMG best models treatment of chronic MG.
- Rapid, effective treatment of long term chronic EAMG suggests that this specific immunosuppressive therapy should work on MG.

Specific Immunosuppressive Therapy With AChR Cytoplasmic Domains in Adjuvant

- Therapy should be robust because many therapeutic epitopes are used.
- Therapy is safe because repeated therapeutic immunization did not induce EAMG.
- Therapy is effective:
 - 6 x 1 mg after acute EAMG prevents development of chronic EAMG
 - 6 x 1 mg during chronic EAMG rapidly reverses it
- Effects are long lasting: treatment that prevented chronic EAMG prevented re-induction 6 months later

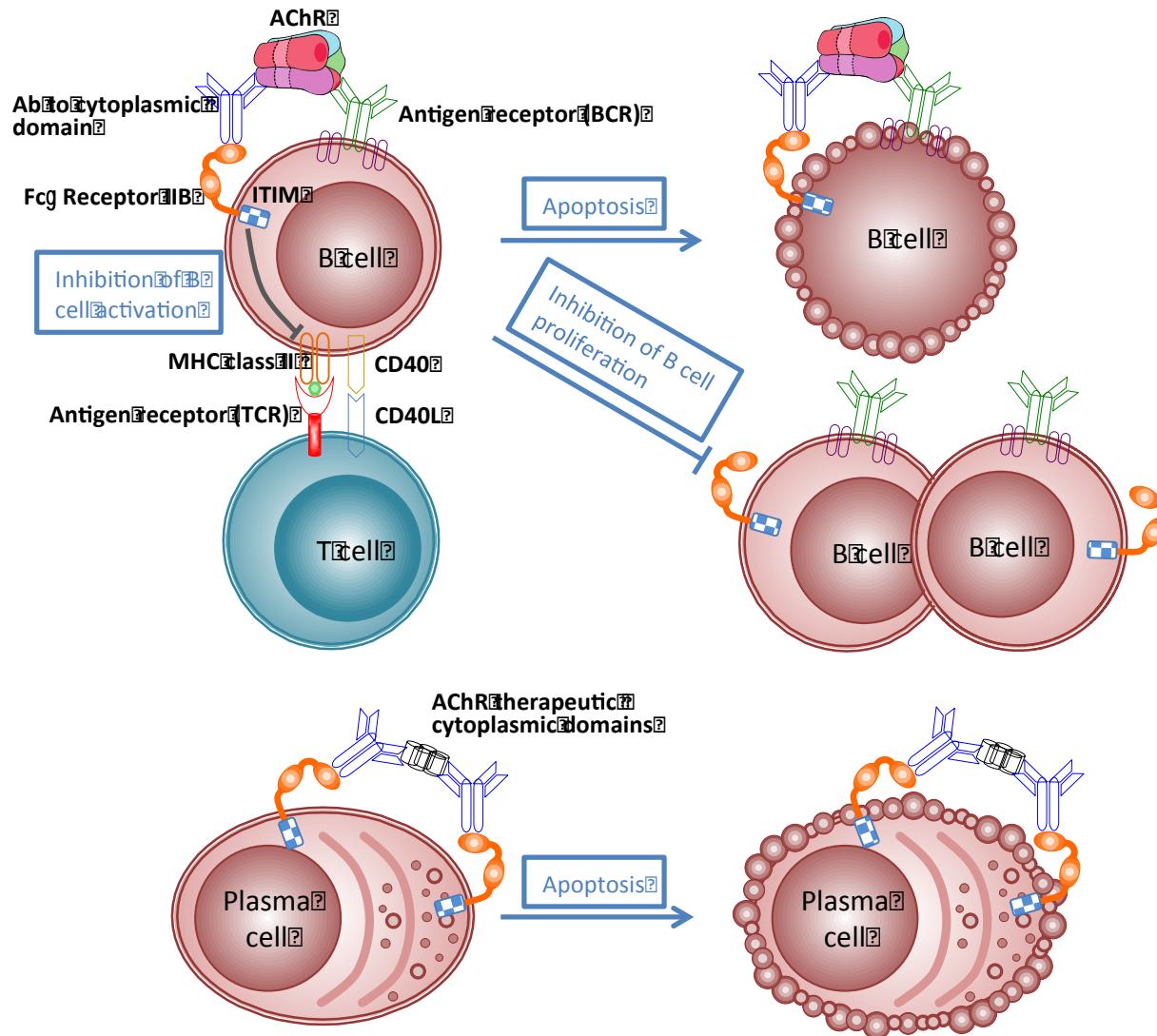
How Does the Therapy Work? (Part 1)

- Antibody-mediated feedback suppression may be involved.
- Antibody to the D antigen is used clinically to prevent hemolytic disease of the fetus and newborn.
- Large amounts of Ab bound to antigen bound to B cell antigen receptors can crosslink Fc γ RIIb receptors on the B cells triggering apoptosis.

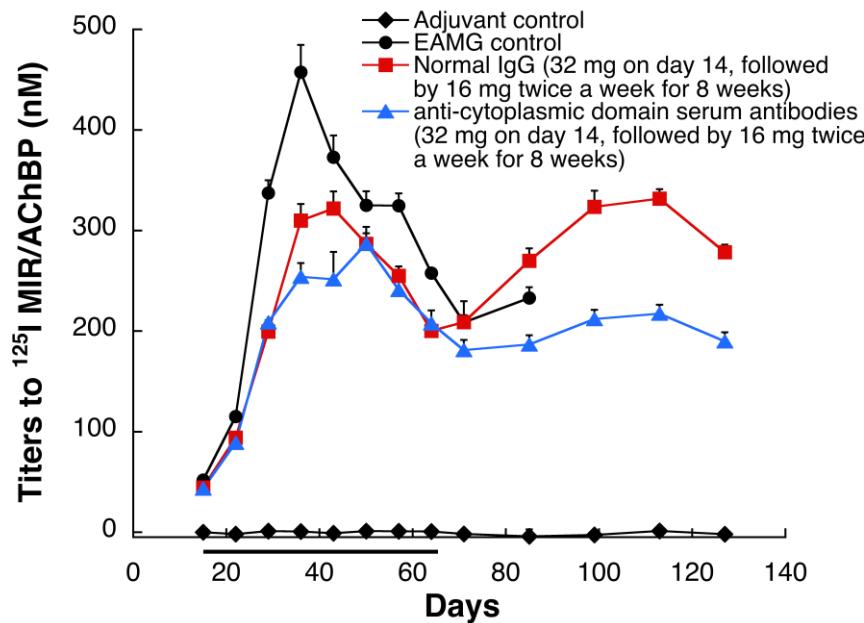
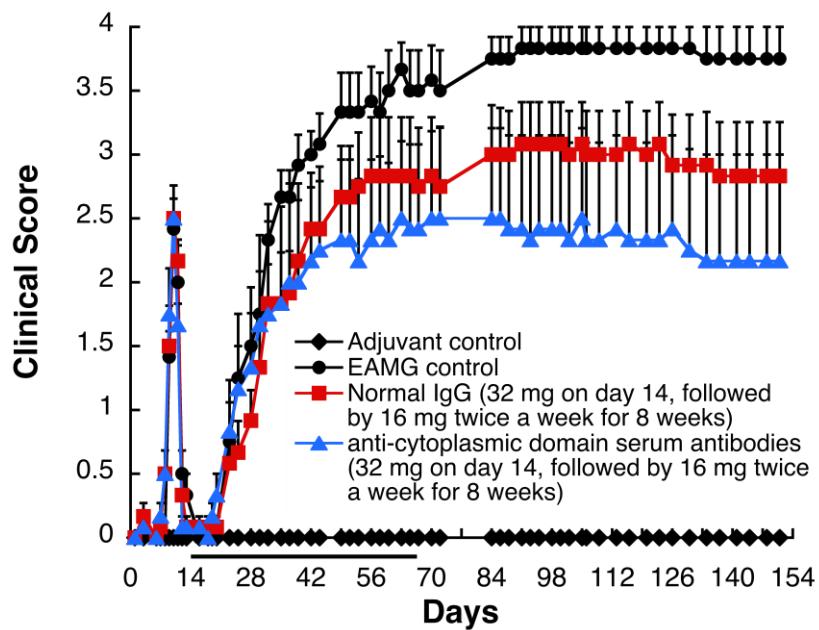
This process is antigen-specific but not epitope-specific.

This explains how antibodies to epitopes on the extracellular surface can be suppressed by antibodies to cytoplasmic domains.

Mechanism of Antibody-Mediated Feedback Suppression



Passive Immunization With Serum Antibodies to AChR Cytoplasmic Domain Reduces Antibody Titer to the MIR and Suppresses Development of Chronic EAMG, But Much Less than Active Immunization



How Does the Therapy Work? (Part 2)

- mAbs to AChR cytoplasmic domains prevent induction and reduce development of chronic EAMG.
- Passive transfer of serum antibodies to cytoplasmic domains is only partially effective at inhibiting development of chronic EAMG. Antigen-antibody complexes provided by repeated antigen doses may be important.
- Induction of regulatory T cells may be important.

Broader Implications

- This basic approach to specific immunosuppressive therapy by immunization with cytoplasmic domains should be effective on antibody-mediated autoimmune responses to any transmembrane protein.

For example:

MG due to Abs to MuSK

encephalitis due to Abs to glutamate receptors